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AN EXPERIMENTAL STUDY ON THE DEVELOPMENT OF THE VASCULAR AREA OF THE CHICK BLASTODERM.[1]

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The question regarding the source of the cell-layer in which occurs the formation of vascular tissue of the opaque area of the bird blastoderm has attracted much attention on the part of students of avian embryology. While the solution of this problem has been sought almost entirely through the study of sections, yet this method of attack is met with many difficulties. It is almost impossible to determine by histological means the exact part played by each of the germ layers in the formation of the vascular tissue of the area opaca. It is not my purpose to discuss the extensive literature that has grown up around this problem; but I shall simply state the two views that are commonly held with reference to the origin of the mesoblast, out of which arises the vascular tissue of the area opaca. According to one view it is derived from the germ-wall, by a delamination; according to the other, it is merely a peripheral extension of the primitive streak mesoblast. If the former view be correct, it is obvious that the germ-wall ought to give rise to vascular mesoblast, irrespective of the presence of the primitive streak mesoblast. In other words, if the primitive streak mesoblast were prevented from growing out over, and coming in contact with the germ-wall, the latter ought still to give rise to vascular mesoblast.

Experiment I.—The experimental test for this proposition is a very simple one, and consists in making injuries on an unin-

[1] Contributions from the Zoölogical Laboratory of the University of Texas, No. 95.

cubated blastoderm just inside the lateral margins of the area pellucida (Fig. 1, *A*). Such injuries effect a fusion between the ectoderm and entoderm,[1] and thus put up a barrier beyond which

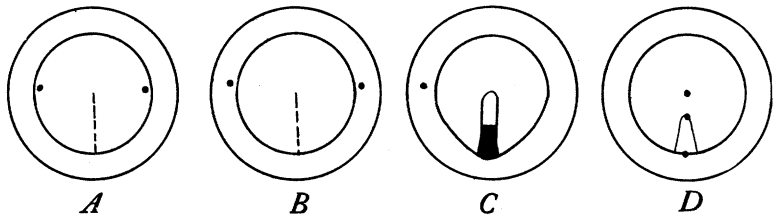


FIG. 1. *A–B* are schemes for operating in Experiments I.–IV. respectively.

the primitive streak mesoblast cannot pass in its peripheral extension.

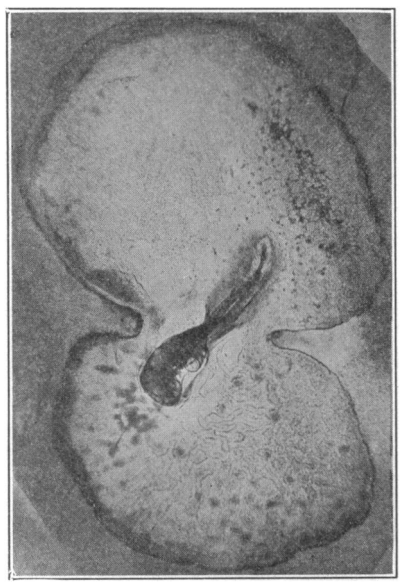
The embryo, as it appears after forty-eight hours of incubation, is shown in Fig. 2. Instead of being circular in outline, as under normal conditions, the vascular area is in the form of a figure eight, the vena terminalis baying in on each side around the obstructing injury-scars. It is easy to see how this result was brought about. The lateral edges of the mesoblast, growing out from the primitive streak, came in contact with the two injuries and at these points the mesoblast was prevented from spreading farther, and so from coming in contact with the germ-wall. There has been also a decided retardation in the spreading of the mesoblast adjacent the injuries. Hence, the transverse diameter of the vascular area, even at the widest points, is only 8 mm., while the longitudinal diameter is 13 mm.

The interesting point brought out by this experiment is made evident when one turns to a study of a section taken across one of the bays (Fig. 3, line *A–B*). The portion of the germ-wall, which lies between the two sections of the vena terminalis, remains undifferentiated (Fig. 4), that is, it has not given rise to vascular mesoblast. It should be noted that it is from this region of the germ-wall that the primitive streak mesoblast has

[1] The methods employed in this work have already been described in the following papers: Patterson, J. T., "The Order of Appearance of the Anterior Somites in the Chick," BIOL. BULL., Vol. 13; "Gastrulation in the Pigeon's Egg—A Morphological and Experimental Study" (in press), *Journal of Morphology*.

been excluded as a result of the operation. The portion of the germ-wall in the region of the section does not differ histologically from that lying beyond the anterior or posterior limit of the vascular area, yet it is at least twenty-four hours beyond the time when it should have given rise to vascular mesoblast (if the first view stated above be correct). Nor does it give any evidence of so differentiating later, even in experiments in which development has been allowed to go on as long as seventy-two hours. This makes it practically certain that in the absence of the primitive streak mesoblast, the germ-wall does not give rise to vascular mesoblast.

Experiment II.—A less conclusive experiment than the preceding consists in making injuries out in the area opaca (Fig.



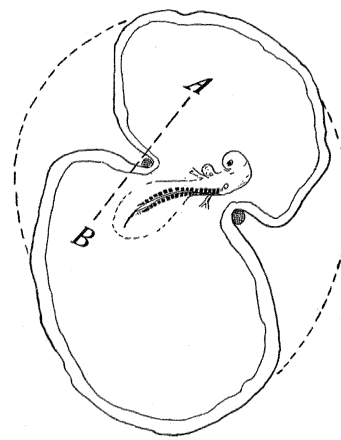


FIG. 2. Photograph of the resulting embryo in Experiment I. The operation was performed on the unincubated blastoderm (see Fig. 1, *A*), and the egg was then incubated for forty-eight hours. The embryo is well developed with twenty-four somites well differentiated. The lateral expansion of the vascular area has been prevented by the two injuries. × 5.8.

FIG. 3. Outline sketch of the embryo shown in Fig. 2. The broken lines at the sides indicate approximately the limits to which the lateral parts of the vena terminalis would have reached had the blastoderm been uninjured. × 4.4.

1, *B*). It gives the same general result, in that the lateral expansion of the vascular area has been retarded (Fig. 5). Sometimes, however, as in the case of the right side of this embryo, the lateral margins of the vena terminalis within the bay grow towards each other and finally fuse, thus leaving the scar of the injury within the margin of the vascular area. In the majority of cases, however, this does not occur.

Experiment III.—Another experimental method of demonstrating that the primitive streak mesoblast is the source of the vascular mesoblast of the area opaca, consists in destroying a given portion of the early primitive streak and then noting the effect on the development of the vascular area. A few of these experiments may be briefly mentioned. If the posterior part

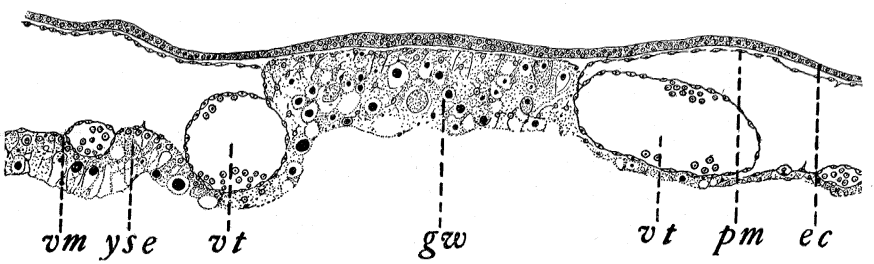


FIG. 4. This is the central part of the section taken in the plane *A–B*, Fig. 3. *vt*, vena terminalis; *ec*, ectoderm; *yse*, yolk-sac entoderm; *pm*, parietal layer of mesoderm; *vm*, vascular layer of mesoderm; *gw*, portion of the germ-wall lying between the two sections of the vena terminalis. Note that it is not differentiated into vascular mesoblast.

of the early primitive streak, together with a small region of the adjacent area opaca,[1] be destroyed, the posterior end of the vascular area is wanting in the later embryo; but the anterior parts of the area are entirely normal. Again, if the anterior tip of the early primitive streak be destroyed, the later embryo shows a deficiency in the anterior part of the vascular area. Finally, if the posterior part only of the primitive streak be destroyed (Fig. 1, *C*), the later embryo lacks the lateral parts of the vascular area which lie opposite the injuries (Fig. 6). In other words, the destruction of any portion of the early primitive streak results in producing a deficiency in the part of the vascular area lying at the level of the injury.

[1] This is done in order to destroy that part of the primitive streak material which overlaps the area opaca at the posterior border of the area pellucida.

Experiment IV.—The foregoing experiment shows conclusively that the differentiation of one part of the vascular area does not depend on the previous development of another part. It does not answer the question, however, as to whether the development of the vascular area is dependent on the progressive differentiation of the embryo. There are a number of ways (experiments) by which one can prevent the development of the embryo, and at the same time show that the primordium for the entire vascular area is laid down irrespective of the differentiation of

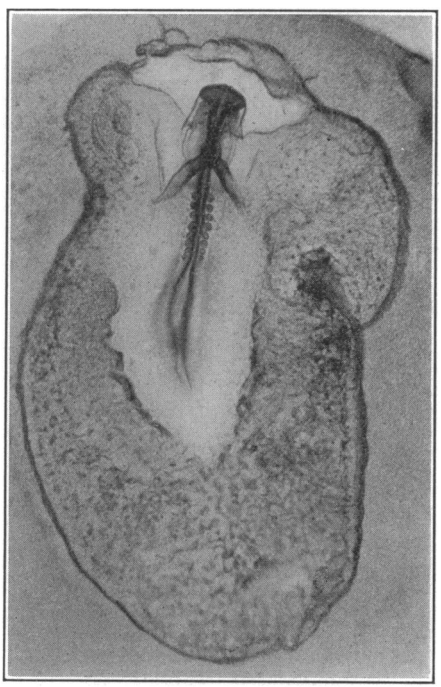


FIG. 5. Photograph of the resulting embryo in Experiment II. The operation was made on the unincubated egg (see Fig. 1, *B*), and the egg was then incubated for forty-nine hours. While development has been greatly delayed, yet the embryo proper is normal, and eight somites are formed. × 12.

the embryo beyond the primitive streak stage. One of the simplest of these is shown in Fig. 1, *D*, and the resulting blastoderm in Fig. 7. Although there is not the slightest trace of the embryo proper present, yet the vascular system of the area opaca

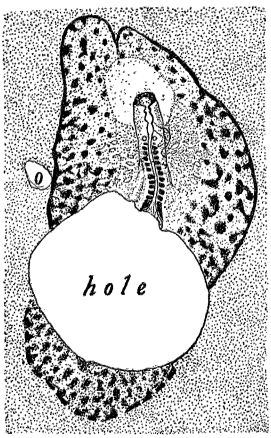
is well laid down, and even the large vessels are seen to pass inwards to the center of the pellucid area. Attempts to develop such blastoderms to the seventy-second hour have failed, from which fact it would seem that after about the forty-eighth hour of incubation the expansion of the vascular area over the yolk is dependent on the propulsion of the newly formed blood-cells through the vessels, that is, on the development of the embryo with its heart.

Discussion.—The results obtained in experiments I. and II. make it practically certain that in the absence of the primitive streak mesoblast the germ-wall does not give rise to vascular mesoblast. Hence, the view that the mesoblast of the area opaca is split off from the germ-wall is doubtless incorrect. There is good evidence, of course, to show that the mesoblast in this area must receive material from the germ-wall. Lillie has pointed out that the mesoderm cells of the area pellucida are void of yolk-granules, while those of the blood-islands "contain yolk-granules of precisely the same character as those of the germ-wall." He states, "therefore, either the blood-islands are derived from the cells of the germ-wall, or cells of the mesoderm growing out over the germ-wall burrow into the latter, engulf yolk-spheres, and reappear in masses as blood-islands."[1] The former of these alternatives is disproved by the result obtained in experiment I.; the latter, therefore, must be regarded as the correct view. In this "feeding" of the mesoderm cells on the germ-wall material during the formation of blood-islands, we doubtless have an explanation for Rückert's[2] contention, viz., that in blood-island formation the primitive streak mesoderm receives elements from the germ-wall; for it is difficult to imagine how it would be possible for the mesoderm cells to take up germ-wall material *en masse* without also including many elements (cells) therein contained. However, the participation of the germ-wall with the primitive streak mesoderm in the development of vascular tissue, must be regarded as secondary (perhaps merely furnishing nutriment), and the power to differentiate blood-islands, there-

[1] Lillie, F. R., "The Development of the Chick," New York, 1908, p. 89.

[2] Rückert, J., "Entwickelung der extra-embryonalen Gefässe der Vögel," "Handbuch der vergl. w. exp. Entw.—lehre der Wirbelthiere," Bd. I., T. 1, 1906.

fore, must be located in the primitive streak mesoderm. To attribute such a power to it is not inconsistent with other facts, for it develops blood-islands in the area pellucida, where there is no germ-wall; but for the rapid and abundant development of islands the mesoderm must come in contact with the germ-wall, where it receives an abundance of food-stuffs. If, however, the germ-



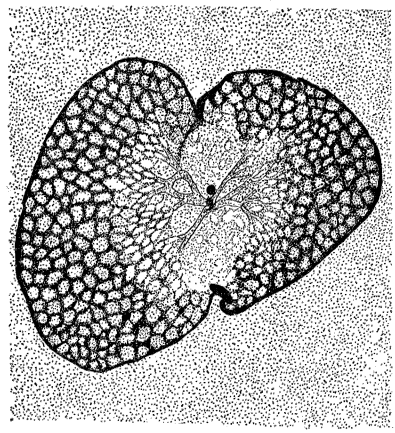


FIG. 6. The resulting embryo in Experiment III. (see Fig. 1, *C*). The operation was made after nine hours of incubation, and the egg was then incubated for thirty-four hours. The region destroyed by the operation is represented by a large hole, opposite to which the vascular area is wanting. Posterior to the hole is a small area of vascular tissue, which doubtless has been formed from the part of the primitive streak material overlapping the area opaca at the posterior border of the area pellucida. The anterior part of the vascular area is slightly asymmetrical, owing to the retardation of the spreading of the mesoblast on the left side, caused by the injury made on this side in the area opaca. × 5.4.

FIG. 7. The resulting embryo in Experiment IV. (see Fig. 1, *D*). The operation was made after about seven hours of incubation, and the egg was then incubated for fifty hours. The embryo is entirely wanting, but the vascular area is well developed, except at the posterior end where its spreading has been retarded by the most posterior injury. The lateral parts of the vascular area consist of a net-work of vessels, and in the region of the pellucid area large vessels pass in towards the center. No heart, however, is developed. × 5.8.

wall elements actually develop into vascular tissue, they must receive their initiating stimulus from contact with the mesoderm.

In addition to supporting the above contention, the results ob-

tained in experiment III. also do away with the necessity for assuming that the vascular area must start as an " anlage " at the posterior border of the area pellucida. There is no doubt but that the first blood-islands appear here, but this is explained by the fact that the primitive streak mesoderm (from primitive plate) is from the first in close proximity to, if not in direct contact with, the germ-wall at this point. The progressive differentiation of the blood-islands from here anteriorly around the inner edge of the area opaca is only an index to the progress made in the expansion of the primitive streak mesoderm.